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Developmental effects of dioxins and related endocrine disrupting chemicals[1]

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Abstract

Alteration of hormones has long been known to affect development. TCDD and related PHAHs modulate the levels of many hormonal systems. Dioxins cause a spectrum of morphological and functional developmental deficits. Fetotoxicity, thymic atrophy, and structural malformations are often noted. Delayed genitourinary tract effects have been observed, and recent studies reported behavioral effects. Highly exposed human offspring have exhibited developmental problems as well. Recently, hormonal and neurological abnormalities have been reported in infants from the general population. The complex alteration of multiple endocrine systems is likely associated with the spectrum of adverse developmental effects caused by dioxin and related compounds.

Keywords: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD); Dioxins; Polychlorinated biphenyls (PCBs); Developmental toxicity; Endocrine disruption; Receptor-mediated toxicity

1. Introduction

Development is a highly integrated process which begins with gametogenesis, proceeds with fertilization, embryogenesis, maturation, and eventually senescence. Developmental effects have traditionally been thought of as birth defects; however, it is critical to go beyond this concept to include the entire life cycle. Development is under hormonal control. A precise integration of multiple endocrine systems is required for all stages in development. The role of estrogens, progestins, gonadotropins, androgens, etc. is well known in regards to gametogenesis and development. The brain, endocrine organs, reproductive organs, and peripheral tissues all contribute to proper functioning of the organism throughout its life cycle.

Given that multiple hormones play critical roles in all aspects of development, it is likely that exogenous environmental chemicals, which can mimic, block, or modulate the endogenous chemical messengers can cause developmental effects [1]. The most important family of such environmental contaminants, for which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) is the prototype, is the polyhalogenated aromatic hydrocarbons (PHAHs), which have been shown to be developmental and reproductive toxicants in multiple species (for recent reviews see [2,3]). Many of these compounds, those which are halogenated in at least four lateral positions, have a common mechanism of action which involves binding to a cytosolic protein, the Ah receptor [4]. This protein functions as a ligand-

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activated transcriptional enhancer [5]. Although it is thought of functioning in an analogous manner to steroid receptors, the Ah receptor is a member of the basic helix-loop-helix (bHLH) family of proteins [6]. As with the steroid receptors, the Ah receptor exists in a multi-protein complex in association with heat shock and other proteins. Upon binding to the ligand, a conformational change results in release of the other proteins and association of the ligand binding subunit, the Ah receptor, with another bHLH protein, ARNT. The ligand-bound heterodimer binds to specific sequences in regulatory regions of DNA, leading to alterations in gene expression. A second method to alter cell signalling has recently been proposed involving activation of protein tyrosine kinases [7]. A schematic of Ah receptor action indicating how ligand binding can alter proliferation and differentiation via two mechanisms is shown in Fig. 1.

A receptor-mediated mechanism of action is characteristic of all hormones, growth factors, and cytokines. It is therefore appropriate to consider dioxin and related chemicals which act via the Ah receptor as environmental hormones. In fact, dioxins are potent mimetics, blockers, and modulators. Almost every hormone system examined has been shown to be altered by dioxin in some cell-type, tissue, or developmental stage [5]. Dioxin may be anti-estrogenic, or require

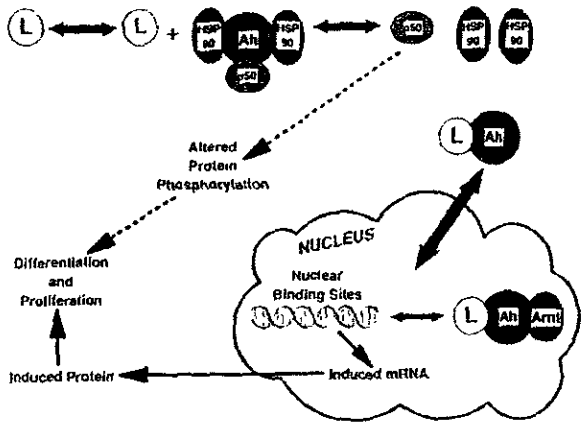

Fig. 1. Schematic of Ah receptor mechanism. L, ligand (TCDD, other dioxins, certain PCBs, etc.); Ah; Ah receptor; HSP90; heat shock protein 90; p50, representative of additional peptides present in multimeric Ah receptor complex; Arnt, aryl hydrocarbon nuclear translocator.

estrogen for its actions. Its effects can resemble both hypo- and hyperthyroidism. Depending on the system, dioxin can antagonize or synergize with glucocorticoids. In addition, dioxin can modulate the actions of insulin, retinoids, growth factors such as TGFα and β, and cytokines such as IL1β. Dioxin can also alter the metabolism of multiple hormones via its induction of both Phase I and Phase II biotransformation enzymes. For example, T_4 is eliminated more rapidly due to induction of glucuronyl transferase activity.

Not all of the PHAHs act through the Ah receptor (Table 1). While Ah receptor interaction appears necessary for all of the laterally substituted dioxins, dibenzofurans, and PCBs, many of the PCBs have difficulty in attaining a coplanar conformation and have extremely limited or no ability to bind to the Ah receptor. In fact, there may be multiple classes of PCBs which have independent, but potentially overlapping, mechanisms and responses [8]. Thus, some of the di-ortho substituted PCBs have phenobarbital-like activity in terms of enzyme induction. Certain PCBs, and especially some of the *p*-hydroxylated metabolites, have estrogenic activity. Sulfonated metabolites have been shown to affect pulmonary function. Some of the lower chlorinated PCBs may inhibit tyrosine hydroxylase activity leading to changes in dopaminergic systems [9] or to changes in calcium homeostasis [10]. Both dioxin and non-dioxin-like PCBs can alter thyroid status, by altered kinetics of thyroid hormones [11] as well as potential interactions with the nuclear thryoid receptor [12]. Recent studies in both humans and rodents have suggested that PHAHs may alter vitamin K metabolism, leading to an increase in bleeding problems in newborns [13]. Prenatal exposure to PCBs has been shown to alter retinoid status [14].

2. Teratogenic effects of TCDD

TCDD and other Ah-receptor ligands are potent developmental toxicants in multiple species (reviewed in [3]). At doses which are not overtly toxic to the mother, dioxin can cause fetotoxicity in the offspring. This is manifested by decreased growth, hemorrhage, and fetal death. Edema is observed in certain species. In

Table 1
PCBs and PCB mixtures

Mixture	% Chlorine	Congener	IUPAC number
Aroclor 1016	21	Dioxin-like:	
1221	21	Coplanar –	
1242	42	3,3',4,4'-Tetrachlorobiphenyl	77
1248*	48	3,3',4,4',5-Pentachlorobiphenyl	126
1254*	54	3,3',4,4',5,5'-Hexachlorobiphenyl	169
1260	60	Mono-orthocoplanar –	
		2,3',4,4',5-Pentachlorobiphenyl	118
Clophen A30	32	Non-Dioxin-like:	
		2,4,4'-Trichlorobiphenyl	28
		2,2',4,4',5,5'-Hexachlorobiphenyl	153

* Mixtures with most dioxin-like congeners.

addition, hypoplasia of the lymphoid organs is often observed at relatively low doses. A recent study [15] has demonstrated that prenatal exposure to PCB #118, which has limited ability to bind to the Ah receptor, leads to hypoplasia of the gastric-associated lymph nodes. Some of the effects on the immune system have recently been shown to be extremely persistent [16]. As previously suggested in studies of prenatal effects on the immune system in mice, thymocyte differentiation is altered in mice prenatally exposed to dioxin [17].

The teratogenic effects of dioxins have been most extensively studied in mice. In fact, as far as obvious structural defects are concerned, until recently there was little evidence of dioxin causing such terata in any other mammalian species [3]. Of course, adverse structural abnormalities induced in birds and fish have been well documented [2]. Dioxin causes hydronephrosis and cleft palate in mice at doses below where any overt fetal or maternal toxicity is detected. Kidney defects have been observed in prenatally exposed hamsters, but these only occur at doses where fetotoxicity is evident. The same can be said for cleft palate in rat pups. In vitro cultures have demonstrated that the embryonic mouse palate is approximately 200 times more sensitive to dioxin-induced clefting than are palatal shelves from rats or humans [18]. Dioxin causes cleft palate by altering the proliferation and differentiation of the medial epithelial cells of the developing palate. These changes are brought about by changes in the balance of various growth factors and their receptors in the target tissue such as TGFα, EGF, and the EGF receptor, and TGFβ1, 2, and 3 [19]. The changes in proliferation and differentiation in the developing palate are also evidenced in the developing urinary tract which inappropriately proliferates leading to blockage of urinary outflow resulting in hydronephrosis in treated offspring (reviewed in [3]). Altered differentiation induced by dioxin has also been reported in the preimplantation embryo [20]. The presence of the Ah receptor has been demonstrated in 8-cell embryos [21]. Both the Ah receptor and ARNT have been shown to be present in the developing palate [22,23] and urinary tract [24]. Doses of dioxin which are teratogenic can be measured in target embryonic tissues within 30 min of maternal exposure [25].

3. Reproductive effects of TCDD

Although dioxin has long been known to be a reproductive toxicant, until recently little effort has been expended in understanding the mechanism of its effects on fertility and reproduction. In part, this has been a result of the focus on overt structural malformations; certain of the effects are also not evident until puberty or even later. Murray et al. [26] demonstrated impaired reproduction in a three-generational study using 10 ng TCDD/kg/day in rats. Recent studies in the laboratory of Peterson and coworkers have examined the effects of prenatal and lactational exposure to TCDD on male rat pups (reviewed in [2]). They noted demasculinization and feminization of the male pups following a single

treatment of 1 μg TCDD/kg on gestation day 15, towards the end of organogenesis. Some of the effects, such as reduced sperm count persisted throughout adulthood. The behavioral changes, originally hypothesized to be due to altered estrogenic or androgenic status, may be due to peripheral effects on the secondary sex structures [27,28]. Based on cross-fostering studies [29], other than the effects on feminizing sexual behavior, decreases in accessory sex organ weights and sperm count, and delayed puberty appear due to prenatal exposure. Similar effects on prenatally exposed male rats and hamsters have been reported by Gray et al. [30]. The developmental exposure to TCDD permanently alters reproductive function in the male offspring of both of these species without any effect on androgenic status. Gray and coworkers failed to observe any change in testosterone or androgen receptor levels in the sex accessory glands or epididymis in the perinatally exposed pups. Nevertheless, the reduction in epididymal and ejaculated sperm appears to be permanent. Although Bjerke and coworkers [27] failed to observe any effect on brain estrogen receptor binding or sexually dimorphic nuclei. CNS involvement has been recently demonstrated by a permanent change in core body temperature induced by this exposure regime in both rats [31] and hamsters (C. Gordon, personal communication).

Gray and Ostby [32] have also examined the effects of prenatal and lactational exposure to TCDD on female rat offspring. In addition to puberty being delayed, structural malformations were present in the external genitalia of the pups. A persistent thread of tissue existed across the vaginal opening in conjunction with partial clefting of the phallus. Because of these alterations, first matings were difficult and often resulted in vaginal bleeding. Ovarian weight was also permanently reduced. Pups prenatally exposed on gestation day 8, rather than 15, had a lower incidence and severity of the genital malformations, but exhibit premature reproductive senescence and an early decline in fertility and fecundity. No changes were noted in sexual behaviors in the female rats or in female hamster pups exposed at the end of organogenesis [33]. Lack of vaginal opening and reduced fertility were also noted in prenatally exposed hamster females.

Similar effects to those observed with TCDD have been noted in studies using the dioxin-like PCB congener #169 [34,35], suggesting that the Ah receptor is involved in the reproductive effects observed. Premature reproductive aging has previously been reported for a commercial mixture of PCBs, Aroclor 1221, which contains few dioxin-like congeners [36]. This mixture also exhibited estrogenic activity, while higher chlorinated mixtures, such as Aroclor 1242, 1254, and 1260, did not. Prenatal exposure in mice to the dioxin-like PCB #77 has recently been shown to reduce the number of germ cells in the ovaries [37], which could potentially lead to premature reproductive aging. Sager and Girard [38] have also reported that perinatal exposure of rats to Aroclor 1254, the mixture that contains the highest concentration of dioxin-like PCBs, led to delayed puberty and decreased fertility in female offspring. Male pups exhibited decreases in accessory sex organ weights, decreased fertility, and increased testes weight [39]. No effects were seen on testosterone levels. Lundkvist [40] observed similar effects of delayed puberty and decreased sex gland weights in PCB-exposed guinea pigs. Overall, the data suggest that many of the effects of PCBs appear similar to those reported for dioxin, suggesting that these effects are mediated via the Ah receptor.

4. Hormonal effects of PHAHs during development

None of the dioxin-like developmental effects appear to be clearly estrogenic or anti-estrogenic. However, a number of the effects recently reported to be associated with exposure to both dioxin and nondioxin-like PHAHs may involve alterations in thyroid hormone levels. Cooke and coworkers [41] have recently shown that transient neonatal hypothyroidism is associated with enlarged testes. In agreement with the earlier study from Sager [39], these investigators [42] have found that prenatal exposure to PCBs also caused enlarged testes and increased testicular sperm count, associated with effects on Sertoli cells. In contrast, as reviewed above, dioxin causes a permanent decrease in epididymal and

ejaculated sperm counts, with little effect on testicular sperm count [30].

Dioxin and dioxin-like PCBs, #77 and 126, cause slight decreases in T_4 levels in weanling rat pups, with no effects on the dams following exposure on gestation days 10–16 [43]. In contrast, much larger effects on circulating T_4 levels were noted in pups exposed prenatally to PCB #118, a congener with limited dioxin-like activity, and PCB #153, a di-ortho substituted PCB with extremely limited ability to bind to the Ah receptor [44]. No effect was seen with the lower chlorinated PCB #28. In addition to changes in circulating thyroxin levels, PCB #118 exposure led to histological changes in the thyroid gland suggestive of elevated TSH levels. Morse et al. [11] have suggested that the PCB-induced decreases in plasma T_4 are due in part to induction of UDP-glucuronyl transferase. The decrease in circulating T_4 appears to be associated with an increase in Type II deiodinase, which converts T_4 to T_3. This increase in a brain-specific form of deioidinase activity may be a result of transient hypothyroidism in the developing brain. Could this play a role in the reported neurotoxicity of PCBs? In fact, Ness et al. [44] have suggested that prenatal hypothyroidism would be consistent with the observed neurobehavioral effects such as spatial learning deficits and altered motor activity. The critical role of thyroid hormones in brain development has been supported by recent studies by de Ku et al. [45] who demonstrated that thyroxine supplementation was able to reverse the decline in choline acetyltransferase activity in hippocampus and basal forebrain of neonatal rats induced by Aroclor 1254, a highly chlorinated PCB mixture containing significant amounts of dioxin-like PCBs. The potential ability of supplemental thyroxine to reverse PCB-induced neurological effects has also been recently demonstrated by Goldey et al. [46]. These investigators have shown that Aroclor 1254 exposure during development reduces circulating thyroid hormone concentrations and causes hearing deficits in rats [47] which are similar to those caused by the potent thyroid antagonist, PTU.

Effects reported on the cholinergic system may be due to the dioxin-like PCBs since neonatal exposure to PCB #77 has been reported to alter muscarinic cholinergic receptors and spontaneous motor behavior in mice [48]. Exposure of developing rats every other day from gestation days 10–20 to PCB #118 or 126 resulted in poorer visual discrimination and higher activity [49]. The potent dioxin agonist, #126, was more effective than #118. These neurobehavioral effects in the offspring occurred in the absence of clinical maternal effects or fetotoxicity. Decreased visual discrimination [50] as well as a decrease in active avoidance learning was also seen following prenatal exposure in rats to the chlorinated PCB mixture, Clophen A30, which contains primarily non-dioxin-like PCBs. Lactational exposure had no effect on these parameters. Of interest are the permanent neurobehavioral effects observed in monkeys exposed both prenatally and lactationally to both Aroclor 1016 and Aroclor 1248 [51]. The deficits observed with the more highly chlorinated mixture on delayed spatial alternation were quite dramatic. Both mixtures caused effects on discrimination reversal learning. The PCB levels in the mother's milk were within the range observed in some human populations. The possibility that neurobehavioral effects of PCBs are, at least in part, associated with the dioxin-like congeners is strengthened by the demonstrated changes in locomotor activity and rearing behavior in rats exposed perinatally to TCDD [52]. In contrast, no effects were observed in prenatally and lactationally exposed male rats in regards to sexually dimorphic behaviors [30].

Neurobehavioral effects of PCBs have been reported in both animals and humans [53]. Whether the observed effects are due to the dioxin-like congeners, the non-dioxin-like congeners, or to the combination is unclear. Children exposed prenatally to heat-degraded PCB mixtures in Japan ('Yusho') and Taiwan ('Yu-cheng') have multiple problems including developmental delays, IQ deficits, ectodermal dysplasia, and growth retardation [54]. Problems at puberty have also been noted in the young men [55]. Behavioral deficits have been noted in children whose mothers had slightly elevated levels of PCBs (reviewed in [53]). Recent studies from the highly exposed Yu-Cheng cohort have noted PCB-induced alterations in auditory event-related P_{300} potentials, suggesting an alteration in cognitive function [56]. Decreased neuro-op-

timality and hypotonicity correlated with the dioxin-like PCBs in infants from within the general population in the Netherlands [57]. Whether this is related to the dioxin-like PCB-associated decrease in circulating T_4 levels in this background population remains to be determined [58].

While it is clear that exposure to high levels of PCBs is associated with clearly adverse effects in the developing offspring, the mechanism of these effects is not clear. The recent observations that differential responses can be measured within the background population, when the population is stratified according to their dioxin-like equivalencies, suggest that subtle effects may be occurring at relatively low levels. The mechanism of such responses remains unknown. However, there is no evidence of enhanced fetal death in populations which may have elevated PCB levels due to increased consumption of PCB-contaminated sport fish [59]. Paternal exposure to TCDD appears to have no effect on pregnancy outcome [60]. Of course, the lack of adverse effects in these two studies is entirely predictable based on animal data.

5. Conclusions

An important message from epidemiological studies is that adverse effects of dioxin can be detected in highly exposed populations, such as those resulting from the rice oil poisonings in the Far East. Recent studies suggest that subclinical effects may also be present within the background population. What does this suggest for future investigations? Firstly, it may not be sufficient to look only for overt alterations in an individual. Instead, the study may need to focus on the distribution of the population. This is a situation reminiscent of that observed with lead. Subtle effects of hormonal alterations during development may put the population into an 'at risk' category, which may only be revealed under stressful conditions or by insightful measurements.

All of the developmental effects discussed above may involve multiple mechanisms. While the dioxin effects, and those of the dioxin-like PCBs, clearly require the Ah receptor, it is essential to understand that the function of this receptor system is similar to that of any hormonal system and involves complex combinatorial interactions. Dioxins initiate a cascade of biochemical changes resulting in alterations in growth and differentiation. How intricate physiological networks and signalling pathways are perturbed by the non-dioxin-like PCBs remains to be determined.

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